Structure and localization of macrophages in lung tissue of asthmatic mice

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Recruitment of macrophages to inflammatory sites of lung tissue and the associated morphological changes play a key role in asthma. We have used waveguide based x-ray holography for tomographic measurements on lung tissue samples. Our goal was to reach the highest resolution possible on the soft hydrated tissue in which macrophages were labeled with commonly used contrast agents to provide insight to the location of macrophages in the tissue. Therefore, a crossed multilayer waveguide [1] consisting of two 59 nm thick crossed multilayers that form a waveguiding channel over a total length of 0.764 mm was precisely aligned with a parallel kinematic robot (SmarAct) to further increase the coherence and resolution of the illuminating beam, which was focussed with crossed KB mirrors. The waveguide creates an effective source of 16 nm FWHM acording to finite difference simulations, enabling spatial resolutions in this range. Previous experiments showed, that on two dimensional (2D) test-structures, resolutions down to below 30 nm can be achieved [2].
As the lung structure is a complicated three dimensional (3D) object, 2D imaging is not enough to locate the macrophages in the tissue. We have previously demonstrated tomographic 3D imaging at the P10 beamline at the level of single isolated cells [3] as well as on the whole organism level [4]. In this case, subcellular structure of barium labeled macrophages in a large piece of lung tissue could be successfully reconstructed at both, a large field of view (FOV) as well as at high resolution for a specific region of interest (ROI) showing clearly the location of barium particles (green) inside the macrophages in the lung tissue. Figure 1 shows a 3D rendering of the reconstructed volume for the large FOV with a specific ROI in the center which is subsequently measured at a higher magnification by simply moving the sample closer to the effective source (see Fig. 2). Further analysis is in progress.

References

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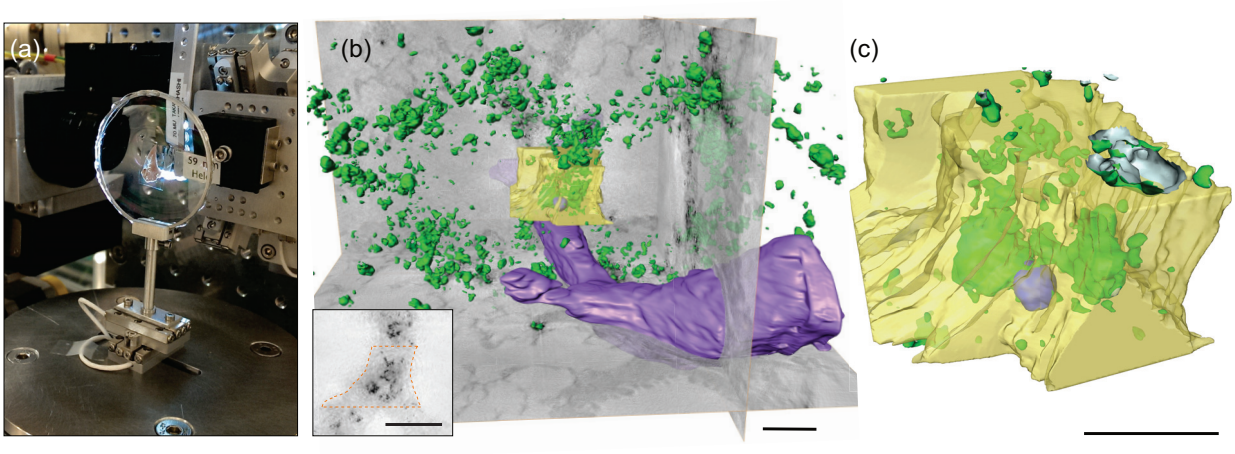

Figure 1: Larve FOV results obtained at P10 - (a) photograph of the sample mounted to the sample holder. (b) 3D rendering of the reconstructed volume, showing three orthoslices together with automatically labeled barium particles (green), a semi automatically rendered blood vesell (purple), a manually labeled bronchial wall inside a ROI (yellow) and the contours of a single macrophage in this ROI (blue). An orthoslice through the area used to segment the bronchial wall is shown in the inset. (c) Zoom into the segmented ROI viewed from a slightly different angle. The same area is measured at larger magnification, shown in Fig. 2. All scalebars denote 50 µm.

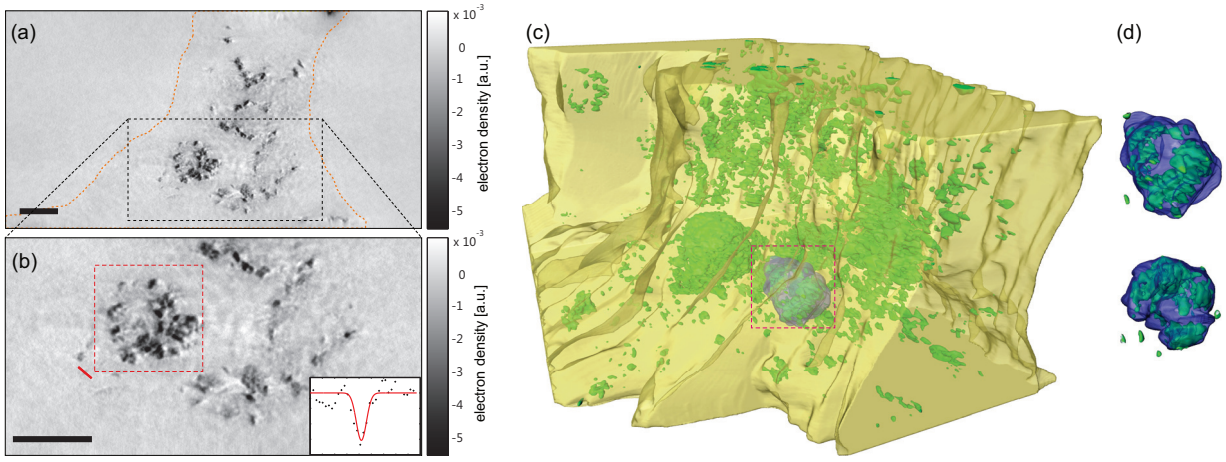

Figure 2: Zoom-tomography results obtained at P10 - The sample shown in Fig. 1 is moved closer to the effective source, resulting in a higher geometric magnification. (a) Slice through the 3D volume showing the area used for segmentation of the bronchial wall. (b) Close up of the area of the dashed rectangle shown in (a). A profile through the solid line is plotted in the inset showing a feature size of 249 nm FWHM. The area marked by the red dashed rectangle shows the cell which is rendered in blue. (c) 3D Rendering of the data showing automatically labeled barium (green), the manually labeled bronchial wall (yellow) and a manually labeled cell outline (blue). (d) Close up of the labeled cell marked by pink dashed lines in (b) and (c) from two different viewing angles showing the internal barium distribution. Scalebars denote 10 µm.